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Influence of the operating conditions and the external mass transfer limitations on the synthesis of fatty acid esters using a *Candida antarctica* lipase

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Abstract

The enzymatic esterification of fatty acids (mainly oleic acid) and ethanol is studied in this work. The reactions are catalyzed by commercial immobilized lipases from *Candida antarctica* (Novozyme 435) and are carried out in a batch reactor at solvent free conditions. The influence of several important reactions conditions as temperature, initial molar ratio, initial water percentage, and enzyme percentage on the equilibrium conversion and the initial reaction rates is carefully analyzed. A non-linear relationship is established between the initial reaction rates and the enzyme percentage. An optimum value of the initial molar ratio is detected for each enzyme percentage and the effect of inhibition by alcohol is confirmed. Under the studied conditions, it is demonstrated that the influence of the external mass transfer resistance on the reaction rate is not significant. Therefore, the experimental data are appropriated for kinetic modeling purposes.
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Keywords: Enzyme; Esterification; Solvent free medium; Fatty acid esters; Immobilized lipases; Multiphase reactors

1. Introduction

Biotechnological production of fatty acid esters with lipases has recently received greater consideration with respect to the traditional methods [1] and is undergoing a rapid development [2–4]. This is due to the mild operating conditions, the degree of purity of the products, and their acceptability in the food industry.

Immobilized lipases can be easily handled and make easier the separation of the catalyst from the reaction mixture and its reuse. The main advantages of using immobilized lipases are, among others, high yields, easy recovery and reusability of the catalyst.

Fatty acid esters have been generally produced by lipases from various sources in organic solvents. But solvent toxicity and cost are a problem for many applications. The major advantages of a solvent free system (SFS) are that the absence of solvents facilitates downstream processing offering significant cost saving and minimizing environmental impact.

The number of publications referred to enzymatic synthesis of fatty acid esters in non-conventional media, particularly in presence of solvents, is numerous [5–9]. On the other hand, the number of articles published about enzymatic synthesis in solvent free systems is considerably lower [10,11].

Habulin et al. [12] have studied the enzymatic esterification of oleic acid and several alcohols using an immobilized *Rhizomucor miehei* lipase. The influence of the different alcohols on the equilibrium conversions and initial reaction rates was evaluated. Peng and Al-Duri [13] investigated the reaction of oleic acid and octanol using the enzyme from *R. miehei* with particular concern to the effects of temperature and octanol concentrations on the initial reaction rates. The use of an immobilized *Candida antarctica* B lipase as catalyst for the direct esterification of oleic acid and ethanol in a solvent free system has not already been reported in the literature, at least to our knowledge.

The purpose of the present study is to investigate the influence of several operating conditions such as enzyme percentage (*E*), initial alcohol/acid molar ratio (*N*), temperature (*T*) and initial water percentage (*W*) on the ethyloleate synthesis using a commercial immobilized lipase from *C. antarctica* B (Novozyme

Abbreviations: Ac, fatty acid (mainly oleic acid); Al, ethyl alcohol; Es, fatty acid ester (mainly ethyloleate); SFS, solvent free system; Wa, water

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Nomenclature

A_i	constant for the component i used in Eq. (6)
B_i	constant for the component i used in Eq. (6)
C_{Ac}	substrate concentration at time t (mol Ac l^{-1})
$C_{Ac}(0)$	initial substrate concentration (at $t=0$) (mol Ac l^{-1})
C_{enz}	enzyme concentration (gcat l^{-1})
C_i	constant for the component i used in Eq. (6)
Da	Damköhler number (dimensionless)
D^0_{AB}	coefficient of solute A in solvent B ($m^2\,s^{-1}$)
D_i	constant for the component i used in Eq. (6)
d_p	particle diameter (m)
E	enzyme percentage, mass of immobilized catalyst/initial mass of fatty acid (%)
i	component i
k_L	solid–liquid mass transfer coefficient ($m\,s^{-1}$)
M	molar weight of the solvent (kg $kmol^{-1}$)
M_m	mixture molecular weight (g mol^{-1})
N	initial molar ratio (mol of alcohol mol of fatty $acid^{-1}$)
r_0	initial reaction rate (mol of fatty acid $min^{-1}\,l^{-1}$)
r'_0	specific initial reaction rate (mol of fatty acid min^{-1} $gcat^{-1}$)
$Re = \left[\frac{\rho_{mix} v_t d_p}{\mu_{mix}}\right]$	Reynolds number (dimensionless)
$Sc = \left[\frac{\mu_{mix}}{\rho_{mix} D_{AB}}\right]$	Schmidt number (dimensionless)
SFS	solvent free system
$Sh = \left[\frac{k_L d_p}{D_{AB}}\right]$	Sherwood number (dimensionless)
T	temperature (°C)
t	time (min)
T_c	critical temperature (K)
T_{cm}	critical mixture temperature (K)
v_A	molar volume of the solute at its boiling temperature ($m^3\,kmol^{-1}$)
V_c	critical volume ($cm^3\,mol^{-1}$)
V_{cm}	critical mixture volume ($cm^3\,mol^{-1}$)
v_t	terminal velocity of the particles ($m\,s^{-1}$)
W	initial water percentage, initial mass of water/initial mass of fatty acid (%)
X_{Ac}	conversion of fatty acid (%)
x_i	is the molar fraction of component i
1, 2	components 1, 2

Greek symbols

$\varepsilon = \left[\frac{V_c^{2/3}}{(T_c M)^{1/2}}\right]$	parameter used in Eq. (7)
$\varepsilon_{mix} = \left[\frac{V_{cm}^{2/3}}{(T_{cm} M_m)^{1/2}}\right]$	parameter used in Eq. (7)
η	external effectiveness factor (dimensionless)
μ_i	viscosity of component i (cp)
μ_{mix}	reaction mixture viscosity (cp)
ρ_i	density of component i ($kg\,m^{-3}$)
ρ_{mix}	reaction mixture density ($kg\,m^{-3}$)
ϕ	association factor of ethanol (dimensionless)

435) in a solvent free system. The influence of these variables on the equilibrium conversion and the initial reaction rates was carefully studied. The effect of the external mass transfer resistance on the initial reaction rate is also evaluated.

2. Experimental

2.1. Materials

The catalyst used was Novozyme 435, a commercial *C. antarctica* B lipase (CALB) immobilized on a macroporous acrylic resin. The product consisted of bead-shaped particles with the diameter in the range of 0.30–0.90 mm. The enzyme was delivered with water percentage of 1.0–2.0% (w/w). It was kindly provided by Novo Nordisk AS (Copenhagen, Denmark). The activity of the catalytic preparation was 7000 PLU/g. One propyl laurate unit (PLU) is defined as the number of μmol of *n*-propyl laurate obtained in the standard test. The activity determination is based on a batch ester synthesis assay where the substrates are 1-propanol and lauric acid; the temperature is 60 °C, and the time at which the activity is reported is 15 min. The ester formation is calculated based on the acid values of the reaction mixture before and after the incubation. The acid values are determined by titration [14].

Alcohols (ethanol: 96.0% (v/v) and 99.8% (v/v)) were purchased from Dorwil (Buenos Aires, Argentina). Fatty acids (mainly oleic acid) were kindly provided by QUIMICAR (Olavarria, Argentina). Standards and other chemicals were provided by Sigma (St. Louis, USA).

2.2. Experimental apparatus and procedure

The experimental apparatus consisted of a batch stirred tank reactor of 250-cm^3 volume equipped with a heat transfer jacket and with an anchor type mixer. (The reactor was specially built by CRIBABB center, Bahía Blanca, Argentina.) To minimize mass transfer limitations the impeller speed was set at 300 rpm approximately. To perform a typical experiment, the reactants were introduced in the reactor where they were heated up to the desired temperature. Once the reaction temperature was reached, the catalyst was added. Samples of 0.10 cm^3 were taken at given time intervals for analysis. The system volume was assumed constant during the reaction because the sampling volume could be considered negligible. When the reaction was finished, the catalyst was separated by filtration, washed with a solvent (ethanol 96.0% (v/v)), and dried. In this way, the lipase was recovered for a next experiment.

2.3. Determination of initial reaction rates

The reaction under study can be expressed as follows:

$$\mathrm{Ac} + \mathrm{Al} \Leftrightarrow \mathrm{Es} + \mathrm{Wa} \quad (1)$$

The initial reaction rates, expressed as the amount of substrate converted per unit of time per unit of weight of immobilized lipase, were calculated from the conversion-time profiles corresponding to the first minute of reaction (5.00% or less of substrate conversion), conditions for which the profiles were found to be approximately linear

$$r'_0 = -\frac{1}{C_{enz}} \left.\frac{dC_{Ac}}{dt}\right|_{t=0} \cong \frac{\Delta C_{Ac}}{\Delta t C_{enz}} = \frac{C^0_{Ac} - C_{Ac}}{(t-0) C_{enz}} = \frac{C^0_{Ac} X_{Ac}}{t C_{enz}} \quad (2)$$

2.4. Analytical procedures

Samples from the reaction mixture were withdrawn periodically. Each sample was dissolved in 20 ml of a mixture of ether/ethanol (50:50, vol.) and analyzed titrimetrically for the residual fatty acid content using an alcoholic solution of potassium hydroxide (normality approx. = 0.01) and carried out at 45 °C with phenolphthalein (alcoholic solution of 1.0%) as an indicator. The substrate conversion (X_{Ac}) was calculated comparing the obtained acid index value with that of the beginning of the reaction.

The results showed in this work are the means of two or more runs. Triplicates were analyzed when it was necessary (specially in the cases where very

low conversions were measured). The vertical error bars corresponding to each experimental data are indicated on the respective figures.

3. Results and discussion

The experiments were selected to study the influence of four operating variables on the reaction rate and the equilibrium conversion. The experimental conditions under which the reactions were carried out are indicated in Table 1.

3.1. Effect of temperature (T)

Experiments at five different temperatures were carried out to analyze its influence on the esterification reaction (Table 1, experiments 1–5). The lower temperature chosen was fixed in 25 °C. Below this temperature the stirring of the reaction mixture becomes difficult because of the high viscosity of the fatty acids. The upper temperature limit was fixed at 65 °C to avoid lipase denaturalization. Fig. 1 shows the conversion of fatty acids versus time for the five temperatures studied. As it can be seen, the temperature has a considerable influence on the reaction rate; in fact, the reaction time required to reach 99.0% of the equilibrium conversion varies from 45 to 10 min when the temperature rises from 25 to 65 °C.

Table 1
Operating conditions for the enzymatic esterification of fatty acids and ethanol

Run	E (%)	T (°C)	W (%)	N
1[a]	10	25	0.20	1.0
2[a]	10	35	0.20	1.0
3[a]	10	45	0.20	1.0
4[a]	10	55	0.20	1.0
5[a]	10	65	0.20	1.0
6	0.10	45	0.20	1.0
7	0.50	45	0.20	1.0
8	1.0	45	0.20	1.0
9	2.5	45	0.20	1.0
10	5.0	45	0.20	1.0
11[a]	10	45	0.20	0.12
12[a]	10	45	0.20	0.25
13[a]	10	45	0.20	0.50
14[a]	10	45	0.20	0.75
15[a]	10	45	0.20	2.5
16[a]	10	45	0.20	5.0
17[a]	10	45	0.20	7.5
18[a]	10	45	0.20	10
19	10	35	0.20	10
20	10	45	0.20	10
21	10	55	0.20	10
22	2.5	45	0.20	0.12
23	2.5	45	0.20	0.25
24	2.5	45	0.20	0.50
25	2.5	45	0.20	0.75
26	2.5	45	0.20	1.0
27	1.0	45	0.20	0.12
28	1.0	45	0.20	0.25
29	1.0	45	0.20	0.50
30	1.0	45	0.20	0.75
31	1.0	45	0.20	1.0
32	1.0	45	4.0	10
33	5.0	45	4.0	10
34	10	45	4.0	10
35	10	45	0.20	1.0
36	10	45	10	1.0
37	10	45	20	1.0

T, temperature; E, enzyme percentage; W, initial water percentage; N, initial molar ratio.
[a] Operating conditions mentioned in Section 3.5.

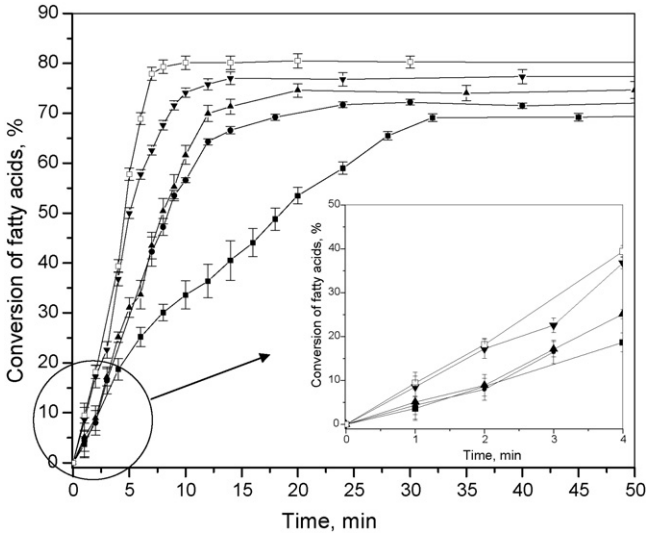

Fig. 1. Influence of temperature on the conversion of fatty acids. (■) T = 25 °C; (●) T = 35 °C; (▲) T = 45 °C; (▼) T = 55 °C; (□) T = 65 °C. Operating conditions: E = 10%; N = 1.0; W = 0.2%.

In agreement with previous esterification studies [15], the equilibrium conversion was found to increase as the temperature increases. After 60 min under reaction, the conversion increased from 69.6% to 80.4% when temperature was increased from 25 to 65 °C.

Fig. 2 shows, on a semi-logarithmic scale, the values of K_c (experimental equilibrium constant based on equilibrium concentrations) versus the reciprocal of the absolute temperature T (K^{-1}). From the slope of a straight line passing through these points, it is possible to calculate the heat of reaction. The

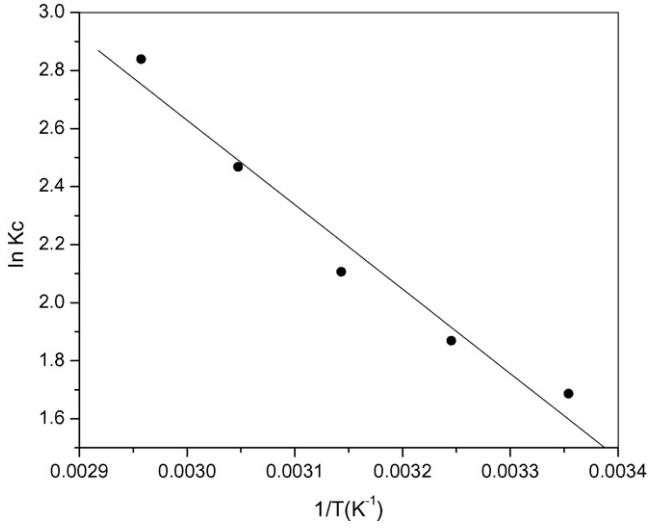

Fig. 2. Experimental equilibrium constant as influenced by temperature. Operating conditions: E = 10%; N = 1.0; W = 0.20%.

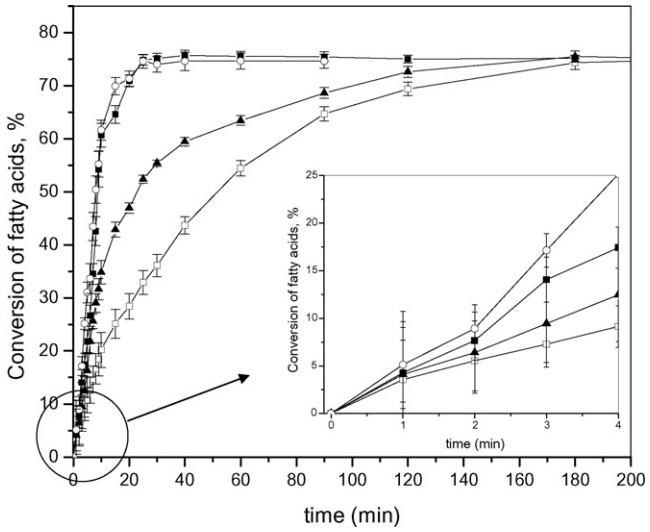

Fig. 3. Influence of the enzyme percentage on the conversion of fatty acids. (□) $E = 1.0\%$; (▲) $E = 2.5\%$; (■) $E = 5\%$; (◯) $E = 10\%$. Operating conditions: $N = 1.0$; $W = 0.20\%$; $T = 45\,^{\circ}C$.

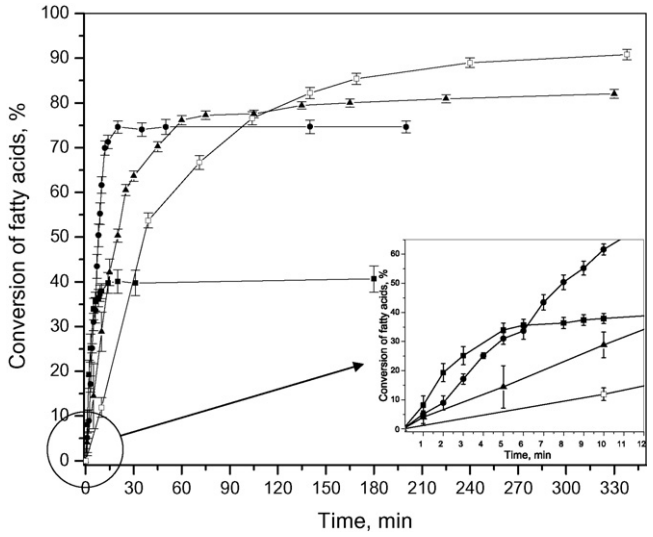

Fig. 4. Influence of the initial molar ratio on the conversion of fatty acids. (■) $N = 0.5$; (●) $N = 1.0$; (▲) $N = 2.5$; (□) $N = 10$. Operating conditions: $E = 10\%$; $T = 45\,^{\circ}C$; $W = 0.20\%$.

value obtained by means of the calculus mentioned before is 20.2 kJ/mol. The heat of reaction for this system, calculated from experimental data of the enthalpies of formation [16], is 24.2 kJ/mol. As it can be seen, both values are in very good agreement. These results suggest that this way of obtaining the heat of reaction is appropriate for the conditions in which the reactions were carried out. However, when the reaction conditions change (e.g. for $N = 10$, experiments 19–21) the calculated value of the heat of reaction also changes significantly (5.48 kJ/mol). This dispersion in the results indicates that the activity coefficients of the reactive species strongly depend on the initial molar ratio (N) and therefore the experimental values of K_c are not always a good estimation of the thermodynamic equilibrium constant, K (T). An analogous phenomenon was reported by Sandoval et al., analyzing a similar reaction but with a different lipase [17]. Consequently, it would be important to develop a thermodynamic model capable to predict adequately the chemical equilibrium within a wide range of operating conditions.

3.2. Effect of catalyst concentration (E)

For the synthesis of ethyloleate, the influence of different amounts of added immobilized lipase on the conversion and the initial reaction rates was studied. To analyze the effect of the catalyst concentration (E = mass of immobilized enzyme/initial mass of fatty acids), six experiments were carried out with E varying from 0.1% to 10% (Table 1, experiments 3 and 6–10).

As it is shown in Fig. 3, the equilibrium conversion is not affected by the amount of catalyst, which suggests that the activity of the components in the mixture do not change with the enzyme percentage.

Before reaching equilibrium conditions, the reaction times needed to get a specific conversion level tend to decrease as the value of E increases. This result is clear within the range $1.0\% < E \leq 5.0\%$. However, it has no sense to employ higher quantities of enzyme than 5.0% due to curves corresponding to $E = 5.0\%$ and 10% in Fig. 3 are almost coincident. This result could be explained taking into account the phenomenon of enzyme agglomeration which was experimentally verified when $E = 10\%$ was selected. When initial reaction rates expressed per unit of volume r_0 are plotted versus the enzyme percentage, it is determined a non-linear relationship in the range of study (data not shown).

An analogous behavior was observed from experiments 32–34 (Table 1), carried out for different initial water percentages (W) and initial molar ratios (N).

3.3. Effect of initial alcohol/acid molar ratio (N)

To determine the influence of the initial molar ratio (N = mol alcohol/mol of fatty acids) on the ethyloleate synthesis, two groups of experiments were selected. A first group was developed at stoichiometric conditions and excess of fatty acids ($N \leq 1$, experiments 3 and 11–14). The second group was carried out in an excess of alcohol ($N > 1$, experiments 15–18). Fig. 4 shows the fatty acids conversion profiles for different N values. As N is increased, it is clear that the conversion of fatty acids progresses more slowly and higher equilibrium conversions are reached.

If the initial reaction rates expressed by unit of catalyst mass are plotted versus N (from 0.10 to 1.0) and for enzyme percentages of $E = 1.0\%$ and 2.5%, maximum values of initial reaction rates are found around $N = 0.80$. After these values a successive decrease on the initial reaction rates appears. The presence of these maximum values around $N = 0.80$ suggests the existence of an optimal initial molar ratio. Others authors [5,13] have reported a similar behavior that has been attributed to inhibition effects caused by the alcohol on the catalyst. Firstly, when alcohol is added to the system and due to its high polarity, it undergoes hydrophilic interactions with the aqueous boundary layer on the lipase surface causing modifications of the protein tertiary structure and therefore enzyme inhibition. On the other

hand, inhibition phenomenon is given by the formation of binary complexes between the free enzyme and the alcohol (or ester) as well as the formation of inactive ternary complexes between the fatty acid or the ester and the enzyme acid-complex [18].

3.4. Effect of water initial concentration (W)

The effect of the initial water percentage on the equilibrium conversion is analyzed in experiments 35–37. The maximum initial water percentage was fixed at 20% and the minimum was 0.20% (corresponding to the water percentage of the absolute alcohol). Commonly, lipases are activated when exposed to an interphase between oil and water [19,20]. However, based on a three-dimensional structure study [21], no interfacial activation has been demonstrated for CALB. The fact that CALB does not require the presence of an oil/water interface to show higher activities is in line with the absence of an optimum value of water concentration found in the present work for the studied range of operating conditions. The presence of water has only an unfavorable effect on the equilibrium conversion. As the initial water percentage increases from 0.20% to 20%, the equilibrium conversion decreases monotonically from 74.4% to 61.0%.

On the other hand, the water is one of the products generated by the reaction. For that reasons and based on the previous results, it seems reasonably to seek different alternatives in order to eliminate the water produced during the esterification. Membrane reactors, reactive distillation, as well as the use of molecular sieves as media of eliminating water from the reaction mixture are promising [22–25].

3.5. External mass transfer effects

The aim of this part of the work is to evaluate the importance of the external mass transfer on the measured initial reaction rates. The Damköhler number was analyzed as an index of which is the controlling mechanism in the system (*Da* = chemical reaction rate/mass transport rate). For some selected operating conditions (indicated by 'footnote a' in Table 1), *Da* was evaluated to calculate the external effectiveness factor (η) by assuming a reversible second order reaction.

The external effectiveness factor, η, was calculated through the following equation [26]:

$$\eta = \left[\frac{1}{2Da}\sqrt{1+4Da} - 1\right] \tag{3}$$

3.5.1. Damköhler number (Da)

The Damköhler number, *Da*, is defined by the following expression:

$$Da = \frac{(\text{chemical reaction rate})_{\max}}{(\text{mass transport rate})_{\max}} = \frac{\text{measured initial reaction rates}}{k_L a C^0_{Ac}} \tag{4}$$

The *Da* number was calculated considering the measured initial reaction rates due to these have their highest values at the beginning of the reaction.

3.5.2. Mass transfer coefficient (k_L)

The solid–liquid mass transfer coefficient required in Eq. (4) is calculated by means of the Frössling correlation [27]

$$Sh = 2.0 + 0.6(Re_p)^{0.5}(Sc)^{0.33} \tag{5}$$

where

$$Sh = \frac{k_L d_p}{D_{AB}}, \quad Sc = \frac{\mu_{mix}}{\rho_{mix} D_{AB}} \quad \text{and} \quad Re_p = \frac{\rho_{mix} v_t d_p}{\mu_{mix}}$$

3.5.2.1. Density of the reaction mixture (ρ_{mix}). The density of the reaction mixtures is calculated from the values of the pure components (i.e. ethanol and oleic acid) and its dependence on temperature is evaluated through the following correlation [28]:

$$\rho_i(T) = \frac{A_i}{B_i[1 + (1 - (T/C_i))^{D_i}]} \tag{6}$$

3.5.2.2. Viscosity of the reaction mixture (μ_{mix}). The viscosity of the solution is calculated by means of the method of Teja and Rice [29]. These authors proposed the following expression:

$$\ln(\mu_{mix}\varepsilon_{mix}) = x_1 \ln(\mu\varepsilon)_1 + x_2 \ln(\mu\varepsilon)_2 \tag{7}$$

3.5.2.3. Diffusivities of the components (D_{AB}). For binary mixtures of solute A in solvent B, the diffusion coefficient D^0_{AB} of A diffusing in an infinitely dilute solution of A in B implies that each A molecule is in an environment of essentially pure B. D^0_{AB} is assumed to be a representative diffusion coefficient even for concentrations of A up to 10 mol% ($x_A \leq 0.10$).

The diffusivity of the components in the reaction mixture is evaluated through the method proposed by Wilke–Chang [30]. These authors proposed the following equation:

$$D^0_{AB} = \frac{1.17 \times 10^{-13}(\phi_B M_B)^{0.5} T}{\mu_{mix} v_A^{0.6}} \tag{8}$$

For $N = 0.1$ (excess of fatty acids) it is considered that the solute is ethanol and it diffuses in fatty acids and for $N = 10$ (excess of ethanol) the solute is fatty acid and it diffuses in ethanol. For the intermediate compositions, that is from $N = 0.25$–7.5, the following lineal relationship between mutual diffusion coefficient and the composition of the mixture was assumed

$$D_{AB} = D^0_{AB} x_B + D^0_{BA} x_A \tag{9}$$

3.5.2.4. Terminal velocity of the catalyst particles (v_t). With the aim of obtaining the Reynolds number for the different conditions of reaction, it is necessary to determine the terminal velocity (v_t) of the catalyst particles. It is calculated assuming that the catalyst particles are moved at their highest velocity relative to the fluid, that is the terminal velocity of the particles in the reaction system.

Viscosity, density, and diffusivity are reported in Table 2 for the experimental conditions selected.

Table 2
Physical properties of the system at selected conditions

Experimental conditions	Viscosity of mixture (cp)	Density of mixture (kg m^{-3})	Diffusivity (m^2 s^{-1})
$N=0.12$ ($T=45\,^\circ$C)	12.9	861	2.59E−10
$N=0.25$ ($T=45\,^\circ$C)	10.1	850	2.87E−10
$N=0.50$ ($T=45\,^\circ$C)	7.49	836	3.06E−10
$N=0.75$ ($T=45\,^\circ$C)	6.00	826	3.20E−10
$N=1.0$ ($T=45\,^\circ$C)	5.03	819	3.31E−10
$N=2.5$ ($T=45\,^\circ$C)	2.79	796	3.61E−10
$N=5.0$ ($T=45\,^\circ$C)	1.83	784	3.78E−10
$N=7.5$ ($T=45\,^\circ$C)	1.48	779	3.85E−10
$N=10$ ($T=45\,^\circ$C)	1.30	777	4.02E−10
$T=25\,^\circ$C ($N=1.0$)	8.07	835	1.96E−10
$T=35\,^\circ$C ($N=1.0$)	6.32	827	2.56E−10
$T=55\,^\circ$C ($N=1.0$)	4.06	811	4.22E−10
$T=65\,^\circ$C ($N=1.0$)	3.33	803	5.32E−10

Figs. 5 and 6 shows the intrinsic initial reaction rate (r^0_{true}) versus the apparent (measured) initial reaction rates (r^0_{obs}), where

$$r^0_{\text{true}} = \frac{r^0_{\text{obs}}}{\eta} \tag{10}$$

It can be seen that values of η between 0.874 and 0.968 are obtained for all the conditions analyzed. Therefore, the external mass transfer effects are not important and could be neglected in order to obtain a kinetic model of the enzymatic reaction.

3.6. Optimal range of operating conditions

As a result of the parametric analysis carried out on the enzymatic esterification of fatty acids in solvent free systems the following optimal range of operating conditions could be outlined:

(1) The temperature should be kept as high as possible (65 °C) in order to get higher reaction rates and higher equilibrium conversion and considering the possibility of thermal deactivation after several reuses.

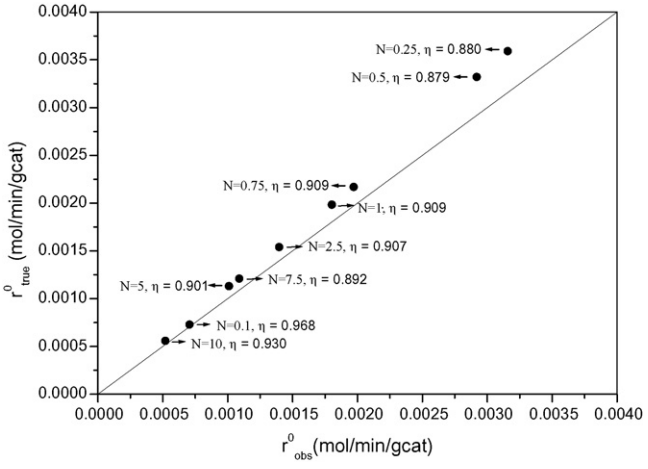

Fig. 5. Influence of the external mass transfer effects for different initial molar ratios. Operating conditions: $T=45\,^\circ$C; $E=10\%$; $W=0.20\%$.

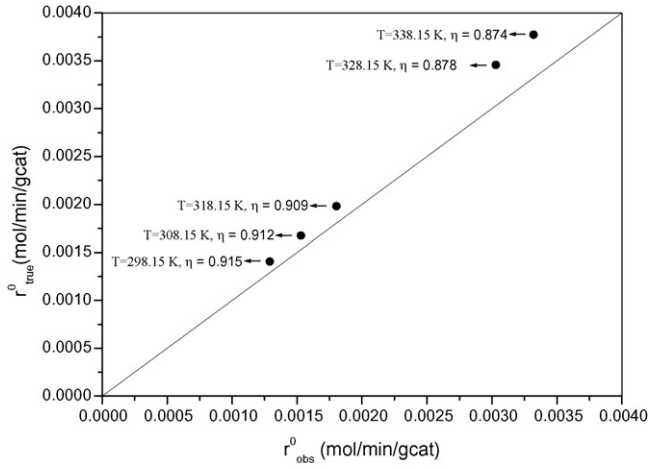

Fig. 6. Influence of the external mass transfer effects for different reaction temperatures. Operating conditions: $N=1.0$; $E=10\%$; $W=0.20\%$.

(2) The optimal enzyme percentage should be in the range of 1.0–2.5%. E values higher than 5.0% could lead to the agglomeration phenomenon already mentioned. Enzymes percentages below 1.0% would reduce the enzyme costs but it could lead to reactions times excessively high.
(3) The initial molar ratio N should be around the stoichiometric value ($N=1.0$). Higher values of N lead to higher equilibrium conversions but lower reactions rates (lower reactor capacities).
(4) The water percentage should be kept as low as possible during the reaction time considering its unfavorable effect on the equilibrium conversion.

4. Conclusions

The enzymatic esterification of fatty acids in a solvent free media is carried out using an immobilized lipase as catalyst. The influence of several operation conditions on the reaction performance was analyzed. The experimental results allowed determining the effect of the temperature, initial molar ratio, initial water percentage, and enzyme percentage on the initial reaction rates and the equilibrium conversion.

A non-linear relationship was established between the initial reaction rates and the enzyme percentage in the range of study. An optimum value of the initial molar ratio was detected, which confirms the existence of an inhibition effect. In addition, the water percentage showed an unfavorable influence on the equilibrium. Shifting the chemical equilibrium to maximize the production rate makes necessary the exploration of novel multifunctional reactor designs.

Under the range of operating conditions studied the external mass transfer limitations show a slight influence on the initial maximum reaction rates. Therefore, the measured reaction rates are appropriated to obtain a kinetic model of the enzymatic system.

Accordingly to the parametric analysis, a range of optimal operating conditions for a large-scale synthesis has been suggested.

To conclude, the enzymatic esterification in a solvent free media is a feasible and interesting way of obtaining fatty acid esters due to the mild operating conditions, and the final product quality. This knowledge will be a useful tool for further kinetic studies and design purposes.

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